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In vitro propagation of Diyarbakır watermelons and comparison of direct-seeded and transplanted watermelon

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Abstract: A rapid protocol using shoot tip explants for micropropagation of Diyarbakır watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] types Sürme, Beyazkış and Karakış was achieved. Shoot tips from 5-day-old in vitro germinated seedlings were cultured on shoot regeneration medium for 3 weeks. The effects of the different concentrations of benzyl adenine (BA) and carbohydrate types on shoot proliferation were examined. The results confirmed that a range of 0.5-1.0 mg/L of BA was almost equally effective in promoting the shoot length of cultures in the 3 genotypes. Sucrose, in the presence of BA, was superior to other carbohydrates for the 3 genotypes studied in terms of the number of proliferated shoots and the average shoot length obtained. The highest percentage of rooting was achieved when medium supplemented with 1.0 mg/L of indole-3-butyric acid (IBA) was used for the 3 genotypes. The highest frequency of acclimatized plantlets for the 3 genotypes was 85%, 85%, and 90%, respectively, in sterile compost, when the shoots of Beyazkış, Karakış, and Sürme were rooted in the IBA-supplemented rooting treatments. The Sürme genotype produced a significantly higher yield and mean fruit weight than the Beyazkış and Karakış genotypes in both direct-seeded and transplanted watermelons. The main soluble sugar of the experimental types was separated, identified, and quantified using high performance liquid chromatography (HPLC). Fructose was found to be the most abundant sugar and was highly detected in Beyazkış for the seeded watermelon and in Karakış for the transplanted watermelon. This demonstrates that in vitro propagation can be used to produce high quality diploid Diyarbakır watermelon for use in breeding lines.

Key words: Watermelon, regeneration, tissue culture, yield, fruit

Diyarbakır karpuzunun in vitro çoğaltılması ve doğrudan tohumdan ve aktarılmış fidelerden elde edilen karpuzların karşılaştırılması

Özet: Sürgün ucu eksplantları kullanılarak Diyarbakır karpuz tipleri Sürme, Beyazkış ve Karakış'ın mikroçoğaltımı için hızlı bir protokol geliştirilmiştir. İn vitro koşullarda çimlendirilmiş 5 günlük fidelerin sürgün uçları 3 hafta süreyle sürgün rejenerasyon ortamında kültüre alınmıştır. Farklı konsantrasyonlardaki benzil adenin (BA) ve karbonhidrat tiplerinin sürgün proliferasyonuna etkileri test edilmiştir. Sonuçlar her 3 genotipte de 0,5-1,0 mg/L aralığındaki BA ortamında sürgün uzunluklarının hemen hemen eşit olduğunu göstermiş ve BA ortamına sukroz eklenmesiyle ortalama sürgün sayısı ve sürgün uzunluğunun diğer karbonhidratlara göre daha yüksek olduğu tespit edilmiştir. Her 3 genotip için en iyi köklenme ortamının 1.0 mg/L IBA olduğu belirlenmiş ve IBA'da köklendirilen sürgünler Beyazkış ve Karakış için % 85, Sürme için % 90 başarıyla steril komposta aktarılmıştır. Sürme genotipinde hem doğrudan tohumdan hem de aktarılmış fidelerden elde edilen ürün verimi ve ortalama meyve ağırlığı, Beyazkış ve Karakış'tan daha yüksek bulunmuştur. Temel çözünür şekerler HPLC (yüksek performanslı sıvı kromatografi) ile kantitatif olarak tespit edilmiştir. Beyazkış'ta tohumdan elde edilen ve Karakış'ta aktarılmış fideden elde edilen karpuzda, en çok bulunan şeker fruktoz olarak tespit edilmiştir. Bu sonuçlarla yüksek kaliteli Diyarbakır karpuzunun in vitro çoğaltılabileceği gösterilmiştir.

Anahtar sözcükler: Karpuz, rejenerasyon, doku kültürü, verim, meyve

Introduction

The wild watermelon has been traced to tropical Africa, Asia, and the North American continent. Watermelon is thought to have been domesticated at least 4000 years ago, and the plant was grown as a crop in the Nile valley (1). However, watermelons were also being cultivated in China, which is today the world's single largest watermelon producer, accounting for about 67,202,000 t of the global production, followed by Turkey at 4,002,000 t, Iran at 3,400,000 t, the USA at 1,793,000 t, and Egypt at 1,485,000 t (2). Although Turkey is not the origin of watermelon, watermelon has been cultivated for years in almost every part of the country. Turkey has valuable watermelon genetic resources, which mainly consist of local genotypes (3). These genotypes are successfully cultivated between the spring and autumn. The southeastern region of Turkey is a microgene center (origin) of the family Cucurbitaceae. Watermelon is the most important and common vegetable species in the region. The major watermelon types produced in Diyarbakır are Sürme, Beyazkış, Karakış, Ferik, and Pembe. However, only the first 3 of these types were commonly grown and became important in the region. These must have been resistant to diseases and insects because of their genetic properties, and these types were also preferred by consumers. Sürme is the most famous type because it is the largest in the country (>50 kg). In addition to market acceptability, Diyarbakır watermelon types have acceptable yield, having adapted to the production areas in southeastern Turkey. Diyarbakır watermelons are also gaining in popularity, as they are ideal for export because of their size and resistance to pests. After harvest, they can keep longer without spoiling, providing a long marketing time. These genotypes are late types, being harvested in the autumn after all other watermelon markets are finished. The Beyazkış ("white winter") and Karakış ("black winter") types were thus named because they can even be eaten in the winter. The genotypes have a thick coat, which helps to conserve them for a long time. This and other recommended properties are important for a breeding program.

The first reports of regeneration from watermelon plants were obtained through the micropropagation of shoot tips and adventitious shoot regeneration (4-12). The earlier reports of regeneration from watermelon cotyledons used both auxins and cytokinins to induce regeneration. Recent reports have shown that benzyl adenine (BA) was sufficient to induce regeneration, and the auxins 2,4-dichlorophenoxyacetic acid (2,4-D), 1-naphthaleneacetic acid (NAA), and indole-3-acetic acid (IAA) promoted excessive callus and reduced shoot formation (13,14). Other cytokinins such as kinetin, 2iP, zeatin, and thidiazuron were all inferior to BA for shoot induction (13). Genotypic variation in regeneration rates is common (13,15). The age of seedling before explant induction varies between laboratories, with 3 to 10 days being common. Several studies looked at mature and immature cotyledons from germinated and nongerminated seeds (14,16,17). The diploid seedlings of Sürme were observed to yield the most regenerative cotyledons 5 days after germination in our laboratory (17). BA without auxin was found to be better for multiple shoot formation from the apical axis of seeds (14,17). Shoot buds maintained and proliferated by routine subculture on Murashige and Skoog (MS) medium with 5-10 mg/L BA retained the ability for subsequent shoot development (17). Somatic embryogenesis from cotyledons of immature zygotic embryos of watermelon has also been reported, but it is limited because of a low frequency of response (13,14,18). Elongated shoots (1-3 cm) could be removed and rooted in vitro. The frequency of shoots per rooting explant was significantly influenced by the concentration of auxin used (11). After 3 weeks on a shoot proliferation medium with kinetin (1-2 mg/L), most of the shoots in the bud proliferation medium were also rooted in vitro. Rooted plants were acclimatized in cell packs and grown in the laboratory at ambient humidity levels for 2 weeks before being transferred to the growth room. Adventitious shoot regeneration systems have been demonstrated as useful for obtaining genetically transformed plants (19,20), but reports of shoot regeneration from watermelon cotyledons are conflicting and it may not be suitable for many commercial cultivars.

Genetic transformation of elite watermelon cultivars (*Citrullus vulgaris*) is a potentially important tool to improve traits such as disease and herbicide resistance without altering the cultivar identity. Stable genetic transformation of *Citrullus* sp. by *Agrobacterium tumefaciens* LBA 4404 has been reported using cotyledon explants (21). A reproducible *Agrobacterium*-mediated protocol, suitable for transferring interesting genes into

different watermelon cultivars, was reported by Ellul et al. (22). However, stable transformation of watermelon has not yet been reported. An adventitious shoot regeneration system has been demonstrated as useful for obtaining genetically transformed plants, but reports of shoot regeneration from watermelon cotyledons are conflicting and this may not be suitable for many commercial cultivars. There are only 2 papers reporting in vitro propagation of Diyarbakır watermelon starting from 5-day-old seedlings with a shoot tip length of 0.5 cm (17,23). Our previous studies on Diyarbakır watermelon consisted of only one type, Sürme.

Watermelon varieties are constantly being changed because of cross-pollination. Therefore, market trends are also changing, and only the elite varieties are acceptable for the markets. Viral diseases are destructive to watermelon and are difficult to control. Several watermelon diseases can cause crop losses, including bacterial fruit blotch, Fusarium wilt, powdery mildew, downy mildew, and gummy stem blight. Viruses such as cucumber mosaic (CMV), squash mosaic (SqMV), and watermelon mosaic (WMV-1,2) also present a problem for growers. Major control strategies for diseases caused by viruses include the use of insecticides to eliminate virus vectors, herbicides to remove alternate hosts for viruses, and genetic resistance (24). Plant biotechnology could be used as a means of introducing resistance to watermelon viruses by inserting segments of viral DNA into existing watermelon germplasm without altering the genetic identity of elite lines. Boyhan et al. (25) and Lecoq et al. (26) identified Plant Introduction (PI) accessions resistant to zucchini yellow mosic virus (ZYMV), and Gillespie and Wright (27) identified PI accessions resistant to WMV. Provvidenti and Gonsalves (28) found, in *Cucumis metuliferus*, that accessions resistant to WMV were also resistant to papaya ringspot virus (PRSV), and that the double virus resistance was controlled by a single dominant gene. However, an efficient in vitro plant regeneration system must be in place before such techniques can be applied. Therefore, the aim of this study was to establish a complete plant regeneration system from the shoot tips of commercial Diyarbakır watermelon genotypes (Beyazkış, Karakış, and Sürme) having breeding importance for genetic research.

Materials and methods

Establishment of aseptic cultures

Watermelon seeds were sterilized for 10 min in 4% NaOCl and rinsed 3 times with sterile distilled water. The seeds were extracted by removing the seed coat, and they were cultured on 50 mL of MS (29) medium, supplemented with 30 g/L sucrose and 7 g/L agar (basal MS medium). The pH levels of all media were adjusted to 5.8 before the addition of agar (Sigma Aldrich) and autoclaving. After germination, the shoot tip explants were used for micropropagation studies. Shoot tip explants from 5-day-old aseptically germinated seedlings were cultured on solidified MS medium containing test concentrations of BA for 3 weeks under a 16-h photoperiod (40 μ mol m^{-2} s^{-1} from cool-white fluorescent lamps) at 25 ± 2 °C. Explants were subcultured to fresh medium of the same composition at intervals of 3 to 4 weeks.

Effects of BA on shoot proliferation

This experiment was conducted to study the effect of different concentrations of BA on shoot organogenesis using shoot tip explants from Sürme, Karakış, and Beyazkış seedlings. All explants were incubated on MS medium supplemented with 30 g/L sucrose, 7 g/L agar, and BA at concentrations of 0.1, 0.25, 0.50, 1.0, 2.0, 4.0, or 8.0 mg/L for 21 days.

Effects of carbohydrates on shoot proliferation and growth

In order to study the influence of various carbohydrate sources on shoot proliferation and growth, 5 subcultured explants were used in experiments with the addition of 3% of sucrose, glucose, maltose, and fructose with 1.0 mg/L BA. After 21 days, the mean number and the mean length of the axillary shoots were recorded.

Effects of IBA on in vitro rooting of the microscions of watermelon genotypes

The explants were transferred to a Magenta GA 7 vessel (4 explants per vessel) containing 50 mL of agar-solidified MS medium supplemented with various concentrations (0.1, 0.5, 1.0, 2.0, and 4.0 mg/L) of indole-3-butyric acid (IBA). The results recorded after 3 weeks of culture included the number of roots and the percentage of the rooted shoots.

Acclimatization

Rooted shoots were rinsed with tap water to remove any adhering medium, then planted in 7-cm plastic pots containing a mixture of the sterilized compost (1:1, peat:turf), covered with a plastic or polyethylene bag to maintain high relative humidity, and placed in a culture room at 25 ± 2 °C (day and night) with a 16-h photoperiod under cool-white fluorescent lights (PAR = 30 µmol m^{-2} s^{-1}) for 3 weeks. In the polyethylene bags, 5 holes (less than 1 mm) were made 1 day after transferring the pots to the culture room, and the number of holes was doubled each day. A 2-week period of acclimatization involved a progressive reduction of the humidity from 95% to 60%. Sterile compost was used for higher plantlet survival rates and plantlets were transplanted to the field at 15-20 days old.

Sugar analyses

The juice of each sample was passed through a 0.45-µm Millipore filter (Sartorius). The HPLC method, modified from the methods of Lee et al. (30), was used to determine the sugar content (fructose, glucose, and sucrose) of the watermelon juice. The column used to analyze the sugar content was a Tessek Polymer Sugar H^+ (250 × 4.6 mm). The software used to interpret the data was Picochrom.

Effects of plant materials in order to determine subsequent effects on crop yield

Both types of watermelon seedlings (direct-seeded and in vitro transplanted) were transplanted to the experiment area in April of 2007 and 2008. The experiment was established according to a randomized design with 3 replicates in rows of 1.5 × 3 m and each parcel contained 20 plants. The watermelon harvest took place at the beginning of August of 2007 and 2008. Watermelons from each treatment were harvested at the time they matured (brown tendril near stem, yellow color development on underside of fruit). Individual fruits were weighted separately and the number of fruits per plot was recorded.

Experimental design and data collection

All of the experiments were conducted using a randomized complete block design. Each treatment was applied to at least 3 replications, with 20 explants per treatment. Significance was determined by analysis of variance (ANOVA); least significant differences among mean values were estimated using Duncan's new multiple range test ($P < 0.05$). Data presented in percentages were subjected to chi-square (χ^2) analysis.

Results and discussion

Influence of BA alone on shoot growth and numbers

There were significant differences in shoot production among the applied treatments (Table 1, $P < 0.05$). All of the treatments tested induced shoot production from the shoot tips cultured. In all of the BA treatments, shoot formation was observed within 5 days in the cotyledon explants of the 3 genotypes studied**.** On the MS medium, highly significant differences were observed in both the mean shoot number and the mean shoot length on day 21 between the tested concentrations of BA (Table 1).

Table 1. Effect of different concentrations of BA on shoot growth of Diyarbakır watermelon genotypes on media containing MS medium.

	BA concentration (mg/L)	The average number of shoots[1] ± SD	The average shoot lenght (cm)[2] ± SD
Beyazkış	Control	1.58 ± 0.14 d	0.73 ± 0.03 c
	0.1	2.58 ± 0.22 c	1.11 ± 0.06 ab
	0.25	4.50 ± 0.41 b	1.29 ± 0.07 ab
	0.5	7.33 ± 0.43 a	1.59 ± 0.07 ab
	1	7.75 ± 0.55 a	1.63 ± 0.14 a
	2	4.91 ± 0.41 b	0.94 ± 0.03 bc
	4	2.83 ± 0.24 c	0.84 ± 0.03 c
	8	1.00 ± 0.00 d	0.00 ± 0.00 d
Karakış	Control	1.75 ± 0.17 ef	0.87 ± 0.04 bc
	0.1	2.66 ± 0.22 de	1.43 ± 0.09 a
	0.25	3.58 ± 0.33 d	1.66 ± 0.08 a
	0.5	6.25 ± 0.46 b	1.57 ± 0.08 a
	1	8.91 ± 0.51 a	1.51 ± 0.06 a
	2	6.58 ± 0.43 b	1.01 ± 0.03 b
	4	4.83 ± 0.36 c	0.93 ± 0.03 bc
	8	1.16 ± 0.14 f	0.67 ± 0.07 c
Sürme	Control	1.66 ± 0.14 f	0.93 ± 0.04 c
	0.1	3.00 ± 0.30 e	1.26 ± 0.08 ab
	0.25	4.91 ± 0.33 d	1.43 ± 0.07 a
	0.5	8.16 ± 0.36 a	1.52 ± 0.07 a
	1	7.16 ± 0.40 b	1.40 ± 0.06 a
	2	5.83 ± 0.36 c	1.02 ± 0.03 bc
	4	3.08 ± 0.37 e	0.88 ± 0.04 cd
	8	1.41 ± 0.14 f	0.62 ± 0.03 d

[1] Data were recorded on the 21st day and present an average of 3 replicates per treatment.

[2] Different lowercase letters in a row above, following any 2 mean values, indicate that these 2 means are not significantly different at the P = 0.05 level of significance according to Duncan's multiple tests.

Explants on medium supplemented with 1.0 mg/L of BA produced the most axillary shoots with the genotypes Beyazkış and Karakış, while the genotype Sürme produced the most axillary shoots with 0.5 mg/L of BA. The results (Table 1) also confirmed that a range of 0.5-1 mg/L of BA was almost equally effective in promoting shoot length of cultures in the 3 genotypes. Significantly shorter and fewer shoots were produced when less than 0.5 mg/L of BA or when more than 2 mg/L of BA was used.

Effect of carbohydrate type

After 3 weeks of culture, the effects of these carbohydrates upon the mean number and the length of the shoots were observed and are shown in Table 2. Sucrose, in the presence of BA, was superior to other carbohydrates for the 3 genotypes studied in terms of the number of proliferated shoots and the average shoot length obtained. The average number of shoots varied between 8.61 and 7.47, respectively, for the Karakış and Beyazkış genotypes with sucrose.

Application of different concentrations of IBA on in vitro rooting

Data presented in Table 3, concerning the different concentrations of IBA, show that all of the treatments were highly significant. The highest percentage of rooting was achieved when MS medium supplemented with 1 mg/L of IBA was used for the 3 genotypes. As the concentration of IBA increased up to 1.0 mg/L, the percentage of root formation increased. In terms of the average root length, the longest roots were obtained when medium supplemented with 1 mg/L (for Beyazkış and Sürme) or 0.5 mg/L (for Karakış) of IBA was used.

Acclimatization

Significant differences occurred in the successful acclimatization of plantlets among the rooted plantlets in the different auxin types (Table 4). The percentage of plants acclimatized to the greenhouse conditions ranged from 45% to 95%. The highest frequency of acclimatized plantlets for the 3

Table 2. Effect of carbohydrate types on shoot growth of Diyarbakır watermelon genotypes.

	Carbohydrate types (3%)	The average number of shoots[1] ± SD	The average shoot length (cm)[2] ± SD
Beyazkış	Sucrose	7.47 ± 0.57 a	1.74 ± 0.14 a
	Glucose	4.76 ± 0.36 b	1.58 ± 0.08 ab
	Maltose	5.52 ± 0.52 b	1.41 ± 0.11 b
	Fructose	5.06 ± 0.43 b	1.65 ± 0.15 ab
Karakış	Sucrose	8.61± 0.76 a	1.69 ± 0.15 a
	Glucose	5.94 ± 0.38 b	1.42 ± 0.12 ab
	Maltose	5.48 ± 0.23 b	1.37 ± 0.18 b
	Fructose	5.15 ± 0.45 b	1.61 ± 0.13 ab
Sürme	Sucrose	8.19 ± 0.54 a	1.59 ± 0.13 a
	Glucose	4.65 ± 0.42 c	1.41 ± 0.09 a
	Maltose	5.63 ± 0.31 b	1.53 ± 0.13 a
	Fructose	4.92 ± 0.37 bc	1.55 ± 0.14 a

[1] Data were recorded on the 21st day and present an average of 3 replicates per treatment.
[2] Different lowercase letters in a row above, following any 2 mean values, indicate that these 2 means are not significantly different at the P = 0.05 level of significance according to Duncan's multiple tests.

Table 3. Effects of different concentrations of IBA on the average rooted microshoot (%) and the average root length of watermelon shoots.

	IBA concentrations (mg/L)	The average root number[1]	The average root length (cm)[2] ± SD	The average rooted microshoot (%)[3]
Beyazkış	Control	0.65 ± 0.04d	0.66 ± 0.03 e	12
	0.1	3.04 ± 0.16 c	1.17 ± 0.11 c	44
	0.5	7.63 ± 0.35 ab	1.59 ± 0.07 ab	71
	1	8.75 ± 0.22 a	1.73 ± 0.14 a	76
	2	6.91 ± 0.22 b	1.44 ± 0.08 b	74
	4	2.43 ± 0.25 c	0.87 ± 0.06 d	51
Karakış	Control	0.72 ± 0.05 e	0.77 ± 0.04 c	14
	0.1	2.66 ± 0.14 d	1.13 ± 0.05 b	42
	0.5	6.55 ± 0.40 b	1.77 ± 0.15 a	67
	1	8.81 ± 0.47 a	1.74 ± 0.07 a	91
	2	6.29 ± 0.31 b	1.26 ± 0.03 b	71
	4	3.83 ± 0.16 c	0.69 ± 0.08 c	63
Sürme	Control	0.86 ± 0.11 d	0.73 ± 0.05 c	11
	0.1	3.47 ± 0.33 c	1.28 ± 0.08 b	57
	0.5	6.17 ± 0.41 ab	1.71 ± 0.14 a	72
	1	7.96 ± 0.24 a	1.86 ± 0.11 a	95
	2	5.24 ± 0.26 b	1.12 ± 0.04b	69
	4	3.58 ± 0.17 c	0.78 ± 0.05c	52

[1] Data were recorded on the 21st day and present an average of 3 replicates per treatment.
[2] Different lowercase letters in a column above, following any 2 mean values, indicate that these 2 means are not significantly different at the P = 0.05 level of significance according to Duncan's multiple tests.
[3] For all types, P < 0.05.

Table 4. Effect of different auxin treatments on plantlet acclimatization in peat.

Genotype	Auxin type used for rooting (1 mg/L)	Viable regenerants[1] (%)	Viable regenerants (%) after transfer to field[2] (%)
Beyazkış	IBA	85	70
	IAA	85	76
	NAA	50	65
Karakış	IBA	85	79
	IAA	75	70
	NAA	45	61
Sürme	IBA	95	89
	IAA	85	79
	NAA	45	72

[1] Data were recorded on day 14 of acclimatization and represent the means of 20 explants per treatment, with 2 repetitions of the experiment. For all types, P < 0.05.
[2] Data were recorded 14 days after transfer to a growth room. For Beyazkış, P > 0.05, and for Karakış and Sürme, P < 0.05.

genotypes was 85%, 85%, and 95%, respectively, in sterile compost when the shoots of Beyazkış, Karakış, and Sürme were rooted in the IBA-supplemented rooting treatments. In the sterile compost, plantlets also tended to resume shoot growth rapidly, with at least 2 pairs of leaves on each plant within 14 days. Subsequent transfer of rooted plants to the field was successful. These plantlets produced as many fruits as the seedlings grown from seed.

Yield of direct seeded and transplanted watermelon

Neither number of fruits nor mean fruit weight was different for the 2 plant materials among Diyarbakır watermelon types; however, the mean fruit number of direct-seeded watermelons of 2 of the genotypes (Karakış and Sürme) was slightly higher than that of transplanted watermelon, whereas only the mean fruit weight of the transplanted Beyazkış watermelon was higher (Table 5). There was also no difference in the number of fruits and the yield between transplanted and direct-seeded watermelons. The Sürme genotype produced a significantly higher yield and mean fruit weight than the Beyazkış and Karakış genotypes in both direct-seeded and transplanted watermelons.

Sugar contents of fruits derived from direct-seeded and transplanted watermelons

The concentrations of fructose, glucose, sucrose, and total sugar contents among the genotypes were found to be statistically significant. Among the detected sugars, fructose was found to have the highest level in all of the experimental genotypes (Table 6). The fructose concentrations were 5.79, 5.32, and 5.15 g/100 mL of juice sample for Beyazkış, Karakış, and Sürme, respectively. The glucose concentration was followed by that of fructose, and sucrose was found to have the lowest level in all of the experimental genotypes among the detected sugars. The concentrations of mean sugar among the genotypes grown from transplanted watermelon were significant, but the mean sugar was approximately the same amount as that of the direct-seeded watermelon.

The cytokinin BA is generally recognized as critical for the production of multiple shoots under in vitro conditions and a number of examples in the literature show the beneficial effect of BA over other cytokinins for shoot multiplication (31). Our results are in accordance with the most recent reports, which showed that BA was sufficient to induce regeneration and that auxins 2,4-D, NAA, and IAA promoted excessive callus and reduced shoot formation (4,14,18). The reports of regeneration from watermelon cotyledons used both auxins (IAA or NAA) and cytokinins (BA, 2iP, or kinetin) to induce regeneration (9-11). In this study, the average shoot length and the number of shoots per explant were highest for all 3 types when the medium was supplemented with BA (0.5-2.0 mg/L) compared to the best kinetin (2.0 mg/L) concentration (Table 1). Our results are in agreement with those of Compton and Gray (4), but are very different from those of Dong and Jia (32), who stated that shoot

Table 5. Number of fruits, yield, and average fruit weight for transplanted and direct-seeded Diyarbakır watermelon genotypes.

Planting method	Direct-seeded			Transplanted watermelon		
	Number of fruits per plant	Yield (kg/m²)	Mean fruit weight (kg)	Number of fruits per plant	Yield (kg/m²)	Mean fruit weight (kg)
2007						
Beyazkış	5.83 a	4.50 b	6.44 b	5.41 a	4.25 b	6.55 b
Karakış	6.08 a	4.85 b	6.65 b	5.66 a	4.40 b	6.48 b
Sürme	4.70 b	6.02 a	12.81 a	4.54 b	5.91 a	11.85 a
2008						
Beyazkış	4.69 b	4.11 b	7.34 b	4.83 ab	4.48 b	6.80 b
Karakış	6.34 a	4.42 b	6.43 c	5.29 a	4.17 b	5.74 c
Sürme	4.16 c	5.69 a	13.30 a	4.33 b	5.65 a	11.51a

Table 6. HPLC qualitative and quantative data of sugars in Diyarbakır watermelon genotypes grown from seed and transplanted plants.

	Fruits grown from direct-seeded watermelons				Fruits grown from transplanted watermelon seedlings			
2007	Sucrose	Glucose	Fructose	Total Sugar	Sucrose	Glucose	Fructose	Total Sugar
Beyazkış	0.76 a	2.40 a	5.79 a	8.95 a	0.62 a	2.44 b	5.45 a	8.51 b
Karakış	0.57 b	2.55 a	5.32 b	8.44 b	0.55 a	2.71 a	5.56 a	8.82 a
Sürme	0.49 b	2.15 b	5.15 b	7.9 c	0.51 a	2.32 b	5.07 b	7.90 c
2008								
Beyazkış	0.95 a	2.26 b	5.34 a	8.55 a	0.63 a	2.97 a	5.23 a	8.83 a
Karakış	0.86 a	2.85 a	4.93 b	8.64 a	0.64 a	2.59 b	5.44 a	8.67 a
Sürme	0.54 b	1.93 c	5.37 a	7.84 b	0.42 b	1.72 c	4.83 b	6.97 b

Values (g/100 mL of juice sample) are expressed as means.

regeneration of watermelon cotyledons was best on MS medium with BA (5 mg/L) and IAA (0.5 mg/L). These differences in the reported results may be related to the genotypes tested. However, BA as the sole plant growth regulator is currently preferred for the production of shoots. The results under discussion indicate that the sole addition of kinetin to the MS basal medium induced not only shoot production, but also root development from cultured shoots for proliferation. In a direct comparison of our regeneration protocol with others (4,7,26), our results gave almost 2 times more new shoots per explant,with the types studied. Similar to Read and Young's (33) results, shoot establishment and shoot proliferation can often be satisfactory with a cytokinin itself (BA), and optimum shoot regeneration has been achieved by adding 1.0-2.5 mg/L of BA as the only plant growth regulator (4,18,34). Although BA itself has generally been reported to stimulate shoot organogenesis in watermelon genotypes, this does not seem to be the case for Diyarbakır watermelon types, because after 3 subcultures, most of the shoots became rosettes. Therefore, the cytokinin BA should be used together with an auxin to stimulate shoot proliferation. The use of IAA and/or IBA in combination with BA caused the fast development of shoots, but NAA caused a drastic decrease in the frequency of explants with shoot buds. Similar to our results, both Compton and Gray (4) and Srivastava et al. (18) detected an inhibition of shoot organogenesis when NAA was added to the proliferation medium. All reports of watermelon shoot organogenesis involved the use of media formulated with full-strength MS medium as outlined by Murashige and Skoog, with additions of sucrose (4,17,18,35). MS medium is very popular because most plants react to it favorably. However, it should be appreciated that MS medium is not necessarily always optimal for growth and development since the salt content is so high. Shoot formation of watermelon improved well on half-strength MS; the growth parameters recorded were the highest values (average shoot length, average number of leaves per shoot, and average number of roots per shoot). Although the medium strength significantly affected the in vitro culture development, the average number of shoots, the average shoot length, and the shoot number per explant were higher when full-strength MS medium was used (Table 2).

The percentage of shoots that rooted ranged from 85% to 95% depending on the genotype and the concentration of IBA used (Table 3). Shoots (10-20 mm in length) were easily rooted in MS medium containing 1.0 mg/L of IBA for the 3 types studied. Similar results were obtained for other watermelon types in media containing 0.2 mg/L of IBA (4) or 0.1 mg/L of NAA (32).

Plantlets were acclimatized in a beaker filled with soilless medium. The genotype and the auxin type used in culture during rooting influenced the ability of genotypes to become acclimatized (Table 4). Poor acclimatization rates for the treatments that contained NAA during rooting may be related to the size of the plantlets at the time acclimatization. As stressed above, NAA caused not only the reduction of shoot development, but also short root development during the rooting stage. Similar to our results, it was reported that the size of the plantlets at the time of acclimatization was important for successful acclimatization of the Dixielee and Minilee genotypes (4). The results of the present study indicate that there was no difference between the amount of yield of the direct-seeded and the transplanted watermelon (Table 5). The highest yield and fruit weight were obtained from the Sürme type. As shown in Table 6, the sucrose, glucose, and fructose, or the total sugar rate, determined in all 3 types that were studied displayed a similar trend in the juice of watermelon fruits grown from direct-seeded and transplanted watermelons. Similar to our results, fructose was found to be dominant and sucrose was lowest in the results reported by Quek et al. (34).

Conclusion

Plants obtained from the protocol described here were normal and true to type. Plants grown in the field were produced from normal male and female flowers that ripened into normal fruit. Fruit yield, number of fruits per seedling, mean fruit weight, sugar contents, and number of seeds per plant were equivalent to plants grown from seed. Therefore, this procedure could be used for the transformation of Diyarbakır watermelon types.

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